

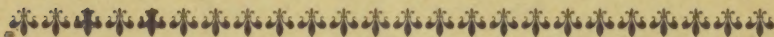
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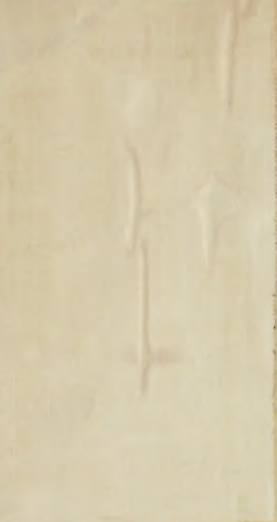


THE IMPORTANCE OF A KNOWLEDGE
OF THE
Diphtheria Culture Test

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I. THE IMPORTANCE OF A KNOWLEDGE OF THE DIPHTHERIA CULTURE TEST.*

II. A PRELIMINARY REPORT ON SOME EXPERIMENTS LOOKING TO THE PRODUCTION OF DIPHTHERIA ANTITOXINE.

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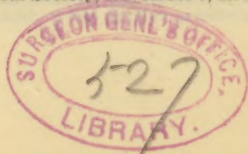
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I.

At this time, when we are hearing so much about the antitoxine treatment of diphtheria, and when the interest in this subject is so great, both in the laity and in the medical profession, it seems highly desirable to emphasize the intimate relations existing between the bacteriological diagnosis and the new treatment.

The blood-serum of a diphtheria-immune animal, or, in other words, the diphtheria antitoxine, is a *specific* for diphtheria, founded upon a prolonged and rational series of highly scientific experiments. The blood-serum of the diphtheria-immune animal is capable of neutralizing the toxins produced by the metabolic activities of the diphtheria bacillus when growing in an artificial culture medium, or when growing in the tissues of a susceptible animal. The blood-serum of the diphtheria-immune animal is not, so far as our present

*The substance of remarks introducing a practical demonstration of the technique of the diphtheria culture test before the Cleveland Medical Society, November 9, 1894.



knowledge enables us to decide, capable of neutralizing the toxins of any other pathogenic micro-organism. The sole condition, then, in which the diphtheria antitoxine is indicated is the disease consequent upon the invasion of the Klebs-Löffler bacillus and the intoxication produced by its peculiar toxins; that is, true diphtheria.

With these facts before us, founded as they are upon the exhaustive experiments of careful investigators, we are in a position to appreciate, to its full extent, the value of any method of diagnosis which will enable us to decide promptly and certainly the nature of the infecting organism in the throat of a patient manifesting the clinical symptoms of diphtheria. Such a method we have in the diphtheria culture test, thanks to the introductory labors of Löffler and his followers in Germany; to Roux and others in France; and to the magnificent work of certain investigators, notably those connected with the health department of New York City, in America.

Since the introduction of the diphtheria culture test we have learned what expert clinicians have always suspected; viz., that the ordinary diagnostic resources of a clinician are not sufficient to enable him to differentiate between a membranous sore throat due to the diphtheria bacillus, and one due to other pathogenic bacteria. This fact has been demonstrated in a startling manner in the investigations prosecuted by the health department of New York City, and one has only to read the voluminous statistics compiled by Drs. Park and Beebe (*Diphtheria and Pseudo-diphtheria, Medical Record*, September 29, 1894, pp. 385-401) to realize the insufficiency of ordinary measures in defining true and false diphtheria.†

The diphtheria culture test is of inestimable value, not only from the standpoint of the new treatment, but also in the proper

†Through the courtesy of Dr. Leick of the health department of this city, and with the assistance of a number of local physicians, I have had the opportunity of recently examining, bacteriologically, the material from 62 cases of throat affection, the majority of which have been reported to the health office as true diphtheria. While this study was undertaken with other objects in view, and while the number of cases is small, still it may be of interest to the local profession to learn that of these 62 cases, only three revealed bacilli in the culture test. Even in these three cases, while the bacilli were the predominating organisms in the twenty-four hours' culture, it still remains for isolation and inoculation experiments to prove that these bacilli are not "pseudo-diphtheria bacilli," since their morphological characteristics are not precisely those of the "typical" virulent Löffler bacillus. The other 59 cases were "micrococcus" throats; that is, micrococci were the predominating bacteria found in the cultures. Of these micrococci the *Streptococcus pyogenes* was the most constant organism; then, occasionally, there was found a culture of the pus staphylococci; and, more rarely still, the *Diplococcus lanceolatus* (pneumoniae). This rather remarkable showing intensifies, it seems to me, the accompanying remarks, and brings very near to our own doors the relation of the diphtheria culture test to diphtheria diagnosis.

sanitary management of membranous throat affections. This, the sanitary aspect of diphtheria diagnosis, has been prominently illustrated in the reports of the New York health department, especially those of Biggs, Koplick, Park, and Park and Beebe; and it forms the foundation of the elaborate system of municipal experiment in this direction, inaugurated in New York.

With the importance of the diphtheria culture test in the diagnosis, treatment, and prophylaxis of diphtheria impressed upon us, we naturally ask how and by whom shall this test be applied? There really seems but one answer to this question—that is, that this test, like many other diagnostic procedures, should be applied by *each individual physician in his own cases*. There are many practical reasons why this should be so, for, notwithstanding the great value of the work in this direction by the New York health department, in which it aims to take from the local physicians the task of applying this test (a work that is bound to be followed ultimately in all our large American cities), it must be evident that, in the present relations of medicine to municipal, state, and national governments, it will be a long time before this kind of work can be of that immediate value demanded in the practical daily work of a physician. The delay incident to the diphtheria diagnosis as practiced in a municipal laboratory, even with the best possible adjustment of the extensive machinery required in a metropolis, is a serious matter to the physician, and especially in its bearings upon the new diphtheria treatment—for, with its truly remarkable results, the antitoxine treatment is only successful as it is applied early in the disease. And even though the work of diphtheria diagnosis, with all that it now means to the physician and to his patient, could be entrusted solely to the hands of the municipal servants in our metropolitan American centres, what are we to do with that vast army of the medical profession and of the public situated outside of the centers of population? In justice to his patient, in justice to himself, and in justice to wonderfully progressive science with which he finds himself associated, the physician whose practice brings him in contact with this class of diseases, must learn to apply the diphtheria culture test himself.

The technique of the diphtheria culture test is not difficult. The preliminary knowledge of bacteriology required for its prosecution is not extensive, and could be acquired by any bright physician with a little study and practice. The special technique of the diphtheria culture test as practiced in America is extremely simple. In fact, the modifications of the technique which have so greatly simplified it and placed it readily within the reach of practicing physicians, are purely products of American ingenuity. In its modified simplicity this diagnostic method could, with a little introductory training, be applied by every individual physician in his practice with the same ease and certainty as an ordinary urinary examination, for instance. The equipment required for this work is, aside from the microscope, inexpensive, when properly selected. An outlay of ten or fifteen dollars would cover the cost of the really indispensable apparatus and material. The time consumed in the actual prosecution of the culture test is no more than that demanded in many of the manipulations of clinical diagnosis in daily use by physicians. The swab obtained from the patient's throat is used to inoculate the culture medium, which is at once set in the improvised incubator and not disturbed for twelve to twenty-four hours. At the expiration of this time the culture is examined microscopically—an operation consuming not more than five minutes' time in the hands of a worker with a little experience—and a *positive diagnosis* is made!

With such objections as the difficulty of the method, the expense of the equipment, and the time consumed in the operation, all set aside, what excuse remains for a physician (especially a young and active man) who does not employ the diphtheria culture test in his individual work?

II.

So much interest has been shown in the immunizing experiments which we have undertaken, and so many questions have been asked concerning the details of the methods, that we have decided, in this preliminary notice, to describe the experiments thus far prosecuted, which have for their ultimate object the production of

the diphtheria antitoxine, according to the methods in vogue with European bacteriologists.

The Organism Employed.—At the outset of these experiments it was necessary to obtain a pure culture of a "typical" and virulent Klebs-Löffler bacillus. This was no easy task, owing to the scarcity of cases of true diphtheria in Cleveland. Fortunately, however, on November 4th, from a Prudden swab submitted by Dr. Charles Gentsch, a typical diphtheria culture was obtained on Löffler's medium.

The clinical history of the case from which the material was obtained was kindly furnished by Dr. Gentsch, to whom I am much indebted for the courtesy. It is, briefly, as follows:

"Gertrude R., aged five years, was first seen October 31, 1894. An examination of her throat revealed a number of small white spots on the tonsils, which, after twenty-four hours, became confluent, and partially covered each tonsil with a membranous patch. The throat lesions were accompanied with febrile symptoms and some general disturbance. The fever lasted but two or three days. The local treatment consisted of liberal and thorough use of hydrogen peroxide every two hours, alternated with douches of a solution of Sihler's tablets. Internally, corrosive sublimate, in solution with tincture of the chloride of iron and chloral hydrate, was employed. Turpentine emulsion and whisky were used as stimulants.

"On the third day the patches had almost disappeared, leaving only a few punctate spots to mark the original points of departure of the membrane. The general condition of the patient was excellent. At this time the swab was obtained from the throat of the patient and at once despatched to the laboratory."

One point of much significance in connection with this case is that, on October 25th, six days before the illness of the girl, Gertrude; her brother, aged six years, was found by Dr. Gentsch to be suffering from a severe attack of croup. The symptoms of this croup had been noted by the parents for two or three days preceding the first examination. The examination made at the first visit by Dr. Gentsch disclosed a few isolated white patches on the tonsils, and on the posterior wall of the boy's throat. The dyspnoea was very marked at first examination, and was not relieved by vomiting. A diagnosis of diphtheritic croup was made, and the vigorous treatment outlined above, was instituted. The laryngeal symptoms gradually increased in severity; operative measures were declined; and the boy died October 27th, with all the symptoms of respiratory obstruction. No cultures were made from this case, and no autopsy was obtained.

In spite of the fact that all precautions against infection were taken during the illness of the boy, and that thorough disinfection was practiced after his death the girl fell ill four days after the death of her brother.

The swab obtained from the throat of the girl was brought to the laboratory by Dr. H. W. Rogers. A tube of Loeffler's serum medium was inoculated from the swab and placed in the incubator. On the following morning, eighteen hours after the receipt of the swab, the inoculated blood-serum was covered with an extensive grayish-white, punctate coating, characteristic of early serum cultures of the diphtheria bacillus. A cover-glass preparation, stained with methyl-violet, 5 B (Grubler), exhibited an almost pure culture of a bacillus that resembled the "typical" Klebs-Loeffler bacillus in size, shape, arrangement, etc. The bacilli were of the long, irregular, more or less beaded variety, with clubbed ends, and other bizarre forms. Aside from these bacilli, a few diplococci were noted in the examination of the first cover-glass preparation. By the use of scratch attenuations on plates of Loeffler's medium, the bacilli were separated from the other organisms. The only other organism isolated on the plates was the *Diplococcus lanceolatus* (*pneumoniae*). After isolating the bacillus, it was studied by the various cultural methods demanded in an exact bacteriological identification of a given species, and it was found to respond typically to each one of these tests. One further test only was necessary to stamp this bacillus a true, virulent *Bacillus diphtheriae*; namely, its pathogenic property. November 11th, 0.5 c. c. of a twenty-four hours' culture of the bacillus in bouillon were injected under the skin of the abdomen of a healthy guinea-pig, weighing 350 grams. Precisely twenty-four hours later the animal died, with certain characteristic paralytic symptoms of early diphtheria intoxication preceding its death. An autopsy was made fifteen minutes after the death of the animal, and all the lesions of experimental diphtheria were found. An intense injection of the blood-vessels under the skin of the abdominal wall; more or less oedema of the subcutaneous connective tissue of the abdominal wall; an intense congestion of the blood-vessels centering in the inguinal regions; a swelling of the inguinal lymph glands with hemorrhagic ecchymoses in their substance; these were the superficial macroscopic lesions observed. The heart was distended with dark and partly clotted blood; the lungs were extremely pale. The liver, spleen and kidneys were paler than normal, somewhat enlarged,

and very friable. The adrenals were enlarged and intensely congested. The vessels of the mesentery and intestines were injected; the mesenteric glands were enlarged and softened; and here and there in the omentum and mesentery hemorrhagic foci were visible. Thus it was demonstrated that the organism isolated in pure culture from this case was a true *Bacillus diphtheriæ*, with all the "typical" characters of this species, and of intense virulence.

The Preparation of the Toxines.—The method of preparing the diphtheria toxines employed in these experiments varies somewhat from that advised by the European bacteriologists. Practically, it represents a combination of the method of Roux and the French bacteriologists, with that of Behring, and Ehrlich and Wasserman, employed in Germany.

On November 18th, 250 c. c. of ordinary, neutral, peptone-bouillon were inoculated from the sixth generation of a pure culture, on Loeffler's serum, of the diphtheria bacillus described above. The bouillon was then set in the incubator and maintained at 37° C. The bouillon was contained in a large, flat-bottomed, Erlenmeyer flask with a side neck. At the expiration of twenty-four hours the side neck of the flask was connected with an aspirating apparatus, and a constant current of moist, filtered air was allowed to pass over the surface of the culture for one week. At the end of the week the aspirating apparatus was disconnected from the flask, which was allowed to remain in the incubator until November 30th. The purity of the bouillon culture was now tested by culture method, and, as it was found to be uncontaminated, it was filtered through a Kinyoun filter. The clear, yellow, germ-free filtrate contained the toxic products of the diphtheria bacilli, or, in other words, the diphtheria toxines. After filtration, one-half per cent. of pure carbolic acid was added to the toxines, which were then set aside in a cool, dark place."

Before proceeding to the next step in the experiment, it was necessary to determine the toxic potency of the finished toxines. This was done by injecting, with a Petri syringe, 0.1 c. c. of the toxines into the subcutaneous tissue of the abdominal wall of a lively guinea-pig, weighing 400 grams. The day following this injection the animal seemed as lively as ever and ate well. Thirty-six hours after the injection the pig was decidedly sick; it sat in

one corner of its cage, refused food, and when disturbed, moved feebly, with the uncertain gait of an animal intoxicated with the diphtheria poison. At the end of forty hours the guinea-pig refused to move when disturbed, and between this time and the expiration of forty-eight hours after the injection, it died. The autopsy exhibited, in an exaggerated form, all the lesions described above, in the experiment with the bouillon culture of the living diphtheria bacilli. Our toxines, then, possessed the required potency.

While these experiments were progressing, the preparation of larger quantities of the toxines was commenced.

The Immunizing Experiments on the Horse.—Thanks to the efforts of my colleagues, and especially to the individual exertions of Drs. H. W. Kitchen and C. B. Parker, a suitable horse for the continuation of our work was provided December 20th. The animal, a mare, about twelve years old, well-nourished, with good digestion, but unfit for road use, is well cared for in Dr. Parker's stable.

On December 20th, the first injection of the toxines into the horse was made. By the use of the graduated pipette of a Petri syringe, 0.5 c. c. of the toxines were injected under the shaven and disinfecting skin of the animal's shoulder. The only suspicious symptoms of constitutional disturbance that we could observe were an exhibition of bad temper on the part of the horse for two or three days following the injection, and its refusal of the morning mess of oats on the second day after the injection. As we made the injection as soon as the animal was brought to the stable and knew nothing of its previous conduct, it was impossible to say what other effect the injection had upon the appetite of the animal. No pronounced elevation of the pulse or febrile symptoms were observed after the first injection. Locally, however, the effect of the toxines was well marked. Within three or four hours after the injection, an area 3 or 4 cm. in diameter, surrounding the needle puncture, became swollen. This swelling increased and persisted for five days, when it gradually subsided. Three days after the injection, a series of blisters appeared over the swollen spot of the injection site. These blisters were still apparent at the second injection.

The second injection of 1.00 c. c. of the toxines was made December 27th, a week after the primary injection. The swelling over the old injection site had entirely subsided, and the second puncture was made a couple of inches to one side of the original one. In both of these little operations, my colleague, Dr. I. J. Kerr, very kindly assisted.

It now remains to gradually increase the dose of the toxines injected into the horse, watching the effects carefully, until an immense dose (250 c. c.) can be tolerated. This, necessarily, will take three or four months; or possibly longer. Barring unforeseen accidents, however, there is no reason why we cannot expect to have, at the end of this time, a diphtheria-immune horse in our midst, whose blood-serum, containing the precious diphtheria antitoxine, will be available for therapeutic purposes.

